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From growth physiology to systems biology

Summary. As it focuses on the integrated behavior of the entire cell, systems biology is a powerful extension of growth physiology. Here, I briefly trace some of the origins of modern-day bacterial growth physiology and its relevance to systems biology. I describe how growth physiology emerged from the foggy picture of the growth curve as a self-contained entity. For this insight, we can thank Henrici, Hershey, Monod, Maaløe, and others. As a result of their work, growth rate is understood to be the unitary manifestation of the response to nutritional conditions and to the control condition for studies on the effect of environmental stresses. For this response to be usefully reproducible, cultures must be in the steady state known as balanced growth. I point out that present-day experimenters are not always aware of this imperative and thus do not always use conditions that ensure the balanced growth of their control cultures. [**Int Microbiol** 2006; 9(3):157-161]

Key words: growth physiology · systems biology · balanced growth

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The development of bacterial physiology

In addition to reading and re-reading *The Microbe's Contribution to Biology*, I have had personal exposure to one of its authors, when I audited the celebrated van Niel course in Pacific Grove, California. Paraphrasing a note I wrote for ASM News (August 2004), the heyday of this course in the 1950s and 1960s coincided with the development of molecular biology. Because this was essentially a microbial science, it seemed like a good idea to many—especially those entering from fields such as physics—to learn something about microbes. Attending the van Niel "finishing school" was an exciting way of doing so. However, a paradox ensued: most of the work in the fledgling science of molecular biology was being done with *Escherichia coli* and its phages. Yet, van Niel barely mentioned these topics in his course. So, why was his course so popular that eminent molecular biologists and their apprentices flocked to Pacific Grove as inexorably as the swallows migrating (far down the California coast) to Capistrano? Mind you, the course was intended for Stanford undergraduates, with the heavyweights and others allowed in only as auditors.

Two answers suggest themselves. One is that the Master was not just a teacher but also a magician. Everyone who took the course reported having come under his spell. Who else but van Niel could keep an audience at the edge of its seats and with pencils poised for eight hours or more of lecture in one day? Who else, on other occasions, would lead the class in a discussion that ended hours later with the conclusion that a certain experiment would solve the problem, only to find that the equipment for just *that* experiment was ready at the back of the room? All this was being witnessed by a wise old sea anemone that had been living in a tank in the laboratory for over 20 years. I can attest to the fact that the van Niel spell can last a lifetime.

The second reason derives from the significance of the Delft School of microbiology, to which van Niel had made stellar contributions. The early days of microbiology ushered

in not just its better-known medical discoveries, but also the pivotal elucidation, by Beijerinck, Kluyver, and their students, of the role of microbes in the cycles of matter in nature. Although the Delft School followed a different development than molecular microbiology, it was held in high esteem. It carried so much authority that taking a course taught by one of its eminent members was assumed to be a sure way to learn about microbes in general. So, an unintended consequence was that many who came to Pacific Grove to get a license to work with *E. coli* left instead with the big picture of the rest of the microbial world. Not a bad bargain.

Publication of *The Microbe's Contribution to Biology* coincided with significant developments in bacterial growth physiology, but as far as I can tell, the Delft School showed relatively little concern for the niceties of bacterial growth. Its main focus was on *which* species were enriched, that is, grew under specified conditions, and *how* they utilized what they had been fed. The assumption was implicit that growth followed some kind of orderly sequence but that the details were not directly relevant to the questions being asked. Fair enough. However, there is fascination in the kinetics of bacterial growth because growth is the most global manifestation of how all cells, bacterial and otherwise, adapt to changes in the environment, how they compete with others, and how they ensure their survival.

A student of microbiology who in the early 1950s wished to get a picture of bacterial growth was hard-pressed to find distinctive guideposts. The focus of the relevant chapters in textbooks, then as now, was the growth curve, with its depressingly unintelligible sequence of phases and, to some early researchers, its hint of life cycles with obligatory stages. Yet, there are fine examples of lucid thinking on the subject of growth from the earliest days of microbiology. One of Pasteur's first three students, Jules Raulin [13], carried out precise quantitative growth measurements with the mold *Aspergillus niger*, demonstrating the requirements of this organism for trace metals. Except for using "sucri candi" as the sole organic compound, Raulin's minimum medium was not too different from M-9 (the chemically defined medium often used for *E. coli*). This medium was based on one devised previously by Pasteur, who Raulin credits with having been the founder of modern microbial nutrition. Despite many such mechanistic examples, the fog surrounding growth physiology remained, fueled by tantalizing but fanciful notions. Thus, the mass of cells was thought to be limited by "biological space." Or, the growth curve of bacteria was seen as sufficiently S-shaped to be inevitably determined by a logistic equation. Not all realized then (or, in some instances, do even now) that the shape of the curve can be altered by as simple a manipulation as changing the size of the inoculum! The focus on the sanctity of the growth curves was unmistakable. In a 1949 review on growth, van Niel [16] stated: "Nearly all that is known about the kinetics of growth of microorganisms has been learned from studies of so-called growth curves." One wonders about his hesitation contained in the "so-called."

Balanced growth

The fog began to be lifted when Jacques Monod reduced the growth response of whole cultures to kinetics analogous to those exhibited by enzymes [7]. The rate of growth was shown to be dependent in Michaelis-Menten fashion on substrate concentration, and the yield was shown to vary with the amount of substrate provided. Monod, however, was soon looking elsewhere: "The study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: it is the basic method of microbiology" [8]. So, how did bacterial growth physiology become a respectable field of research?

As is often the case, subsequent work was facilitated by a clear definition. Campbell [1] defined growth in a steady state, or "balanced growth," as the condition in which all cell constituents increase by the same proportion over the same interval of time. This definition dignified what was previously just a so-called "phase" in the growth curve (the exponential phase) by a physiologically meaningful generalization. The difference between "exponential phase" and "balanced growth" is the difference between watching apples fall and thinking of gravity. Many workers had realized the importance of growth at a steady state, but Campbell's novel term and precise definition helped remove the aura of immutability from the growth curve. No longer was the growth curve sacrosanct. It was clear that periodic dilutions extended the period of balanced growth for as long as the experimenter desired. In the early 1950s, a novel way to manipulate the growth rate was introduced—continuous culture growth in the chemostat [9,12]. A culture in balanced growth, either in batch culture or in a chemostat—it was realized—was the only readily reproducible condition in which to study physiological phenomena.

In the mid 1950s, work in Ole Maaløe's Copenhagen laboratory led to the generalization that the macromolecular composition of bacteria is a monotonic function of the growth rate [14]. The faster the growth rate, the higher the cellular concentration of DNA, RNA, and total proteins. Cells growing (at a particular temperature) at the same rate in chemically different media have the same overall concentration of nucleic acids and total protein. From these data

emerged the concept that physiological states are imposed on the cell by its rate of growth and that a bacterium is not defined by a unique composition. Rather, this is highly variable and dependent on the nutritional environment. I quote again the Spanish philosopher José Ortega y Gasset: "Yo soy yo y mis circunstancias" ("I am I and my circumstances").

With the knowledge that the growth rate predicts the cellular profile, it became possible to determine how cells behave during the transition between physiological states [6]. Thus, shifting cultures from a medium that affords a slow growth rate to one that leads to a higher rate results in a rapid acceleration of ribosome synthesis. The converse, going from fast to slow growth, imposes a long lag required for the synthesis of biosynthetic enzymes that were repressed in the rich medium. Both patterns could be partly understood in terms of the partitioning of the transcriptional and translational apparatus between synthesis of the repressible biosynthetic enzyme systems and making the protein synthetic system. This was called by Maaløe "passive control" regulation [5].

The study of shifts between rich and poor media and vice versa dealt a final blow to the growth curve as a mystical entity [6]. The lag phase could now be likened to a nutritional shift-up and the stationary phase to a series of progressive shifts-down. The mystery of the lag had been partially lifted as early as 1938 by Hershey [4], who showed that, using fully viable cultures in stationary phase, the cell mass increased immediately upon inoculation into fresh media, but that cell division lagged behind. The impact of this work was not as great as that of the Copenhagen reports 20 years later, one reason being that Hershey did not attempt to correlate his finding with Henrici's classic report [3] on the changes in bacterial size throughout the growth cycle. The papers from Copenhagen made this connection and presented a unitary explanation. For an analysis of the origins and meaning of this works, see the article by Cooper [2].

The limitations of the Copenhagen study of growth physiology were real because molecular and genetic tools were in their infancy. Francis Crick understood this disquietingly early. When I visited him at the Cavendish labs in 1958, he told me: "Congratulations! You people started a new field, but it will end with what you did." As a discipline, our kind of growth physiology came close to passing from inception to oblivion in a single leap! In a narrow sense this was true, but more broadly speaking the physiological focus on the growing cell had contributed both a needed counterpoint and a complement to molecular reductionism. Due to the insight of many people, notably Maaløe, Neidhardt, Magasanik and others, the work was extended to the concerns of the day about the relationship of nucleic acids to protein synthesis, as seen from a cellular point of view. Thus, a window was opened to a molecular mechanism, and Crick turned out to be wrong, at least in part. For a lucid manifesto of this position, see the commentary by Frederick C. Neidhardt [11].

Should the growth conditions of a culture still be a focal concern for systems biology? Absolutely! With so many investigations of the responses to physiological stresses, the base line or "control" culture retains a central position. Basically, this is an issue of reproducibility, ensuring that an experiment can be replicated in one's own lab and elsewhere. Deviate from the steady state condition, and samples taken at successive times will be different. Nonetheless, in practice, attention is not always paid to experimental details that ensure balanced growth.

Equal attention must be paid to the reproducibility of the culture medium used. Complex media, such as LB (Luria–Bertani), are made with batches of undefined constituents, such as peptone and yeast extract. These vary from batch to batch, although the differences may be small. One way to avoid the uncertainties of complex media is to use a synthetic medium, such as MOPS (3-[*N*-morpholino]-propanesulfonic acid) [10]. By adding one or another carbon source to MOPS, one can choose a "minimal" medium or, if enough defined supplements are added, come close to obtaining the growth rates in rich broths.

A practical note

Not infrequently, articles published in the literature include only a casual mention of how the cultures were grown. The inoculum used is sometimes so large that the culture goes from a lag to a stationary phase with only a brief interlude in exponential growth. A 1:100 inoculum from an overnight culture may seem small, but, for the usual enteric bacteria, it means that the experiment was started with some 10^7 cells/ml, an inoculum much too large for an extended period of growth. To attain balanced growth requires at least a 1:10,000 dilution of such an inoculum, which can readily be done by periodic dilutions of the culture. An indication of indifference to proper growth conditions is the use of terms such as "mid-log." This is nearly meaningless. It is a mid-point of what? The lower point used for determining this value depends entirely on the size of the inoculum used, so one investigator's "mid-log" could be someone else's lag or stationary phase. A modern source of error may be introduced by the use of multiple-well plates and automatic measurements of the optical density of the culture. Proper aeration, needed for maximal growth of facultative bacteria and essential for strictly aerobic bacteria, may be hard to achieve with the par-

ticular geometry of small wells. In addition, frequent measurements require stopping the shaking of the cultures. I propose that, mundane as it sounds, the growth conditions and a growth curve should be published in detail in the Supplemental Materials section of papers that describe growing bacteria.

Systems biology

How is bacterial growth physiology of old days connected to systems biology of today? Both historical and conceptual threads are clearly visible. In fact, some consider system biology as an all-embracing view of cell physiology, or, if you wish, a continuation of the escape from biochemical reductionism. The questions asked in the old days—how many macromolecular components are in a cell, how rapidly are they made, and how do they interact to result in cell growth—are the same as those being asked now. The difference is that now we have tools to probe these questions in a detailed, extensive, precise, and rapid manner. Yet, even modern methods have a direct connection with the old ones. An example is the proteomic measurement of growing and stressed *E. coli*, first done on a large scale in Neidhardt's lab [17]. The early impetus for this work was to determine the number of proteins made at different growth rates, soon augmented by looking at the effects of physiological stresses. But this aim was quickly replaced when Neidhardt realized that up to this point bacterial studies had been guided largely by *what the investigator* thought interesting, useful, or potentially vital to the cell. Instead he saw that new methods—notably, two-dimensional gel electrophoresis—of surveying the global production of proteins enabled the investigator to put the ball in the microbe's court and discover *what the cell* deemed important. Seeing the proteins made not just at different nutritionally imposed growth rates, but also at different temperatures and when the cells are subjected to various chemical and physical stresses, led to a broadened appreciation of the whole cell as a dynamic system replete with an expanded universe of rules and relationships governing physiology and metabolism.

The value of systems biology lies in its ability to create predictive models, something that has been achieved to a considerable extent with yeast and is currently being carried out with bacteria. We are beginning to get a multidimensional view of the complex network of interactions that lead to the growth of a cell. As ever, the experimental basis for this work has to be growing cells under reproducible and readily assayable conditions—in other words, using cultures in balanced growth as the baseline condition. This is but one of the concepts that systems biology has inherited from growth physiology. Elsewhere, we have enumerated some other caveats that need to be kept in mind when probing into cellular "systems" [15].

Aficionados of balanced growth, such as myself, are sometimes reminded that this condition is unusual in nature. Indeed, most environments are changeable, allowing, at most, short spurts of unhindered growth. But that is not the cells' fault. Most planktonic cells—and possibly many sessile ones—have the urge to grow as rapidly as conditions permit. The experimenter who provides conditions that permit balanced growth is doing no more than letting cells put into action this fundamental yearning.

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De la fisiología del crecimiento a la biología de sistemas

Resumen. Al centrarse en el comportamiento integrado de la célula en su totalidad, la biología de sistemas es una poderosa extensión de la fisiología del crecimiento. En este estudio se indican brevemente algunos de los fundamentos de la fisiología moderna del crecimiento bacteriano y su importancia en la biología de sistemas. Se describe la forma en que la fisiología del crecimiento emergió como entidad autónoma del cuadro brumoso de la curva de crecimiento. Podemos agradecer en todo esto las percepciones que modelaron Henrici, Hershey, Monod, Maaløe y otros. Gracias a sus trabajos se ha entendido que la velocidad de crecimiento es la manifestación unitaria de la respuesta a las condiciones nutricionales y es la condición que rige los estudios sobre el efecto del estrés ambiental. Para que todo ello pueda ser útilmente reproducible, los cultivos tienen que estar en la fase de crecimiento sostenido que se conoce como crecimiento equilibrado. Queremos destacar que los experimentadores de hoy en día no siempre tienen conocimiento de este imperativo y no siempre emplean las condiciones que aseguran el crecimiento equilibrado en sus cultivos de control. [**Int Microbiol** 2006; 9(3):157-161]

Palabras clave: fisiología del crecimiento · biología de sistemas · crecimiento equilibrado

Da fisiologia do crescimento à biologia de sistemas

Resumo. Ao estabilizar-se no comportamento integrado da célula na sua totalidade, a biologia de sistemas é uma poderosa extensão da fisiologia do crescimento. Neste estudo indicam-se brevemente alguns dos fundamentos modernos da fisiologia do crescimento bacteriano e sua importância na biologia de sistemas. Descreve-se a forma em que a fisiologia do crescimento emergiu como entidade autônoma do quadro brumoso da curva do crescimento. Podemos agradecer isto às percepções que Henrici, Hershey, Monod, Maaløe e outros modelaram. Como resultado dos seus trabalhos, entende-se que a velocidade de crescimento é a manifestação unitária de resposta às condições nutricionais e às condições de controle para aqueles estudos sobre o efeito do estresse ambiental. Para que tudo isso possa ser utilmente reproduzível, os cultivos têm que estar na fase estacionária, conhecida como crescimento equilibrado. Queremos destacar que os pesquisadores atuais nem sempre têm conhecimento deste imperativo e nem sempre empregam as condições que asseguram o crescimento equilibrado nos seus cultivos controle. [**Int Microbiol** 2006; 9(3):157-161]

Palavras chave: fisiologia do crescimento · biologia de sistemas · crescimento equilibrado